MBMG
Metabarcoding & Metagenomics

Metabarcoding and Metagenomics 8: 469–488 (2024)
DOI: 10.3897/mbmg.8.135461

Research Article

Assessing the impact of tidal changes on fish environmental DNA metabarcoding in temperate and tropical coastal regions of Japan

Shin-ichiro Oka[1], Tetsuya Sado[2], Masayuki Ushio[3], Masaki Miya[2]

1 *Okinawa Churashima Foundation, Okinawa 905-0206, Japan*
2 *Natural History Museum and Institute, Chiba, Chiba 260-8682, Japan*
3 *The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR*

Corresponding authors: Shin-ichiro Oka (okashin1ro@gmail.com); Masaki Miya (masaki_miya@me.com)

Abstract

Environmental DNA (eDNA) analysis is a valuable tool for detecting species presence in coastal environments. However, its effectiveness can be influenced by factors such as diffusion and degradation after being released by organisms. Understanding eDNA dynamics in response to tidal currents is pivotal, especially in the intertidal zone where water movement is pronounced. This study assessed the impact of tidal movements on fish eDNA metabarcoding across two regions separated by a 10-degree latitude difference: the temperate Boso Peninsula (35°N) and the tropical Motobu Peninsula (25°N). Over two days, we conducted four rounds of water sampling at three sites within 20 km of each Peninsula's coast during substantial tidal differences (1.5–2 m), covering both outgoing and incoming tides. Fish eDNA metabarcoding analysis revealed notable differences in fish community compositions between the two Peninsulas, with statistically significant variations amongst the three sites within each Peninsula. At each site, comparison between outgoing and incoming tides showed significant differences at five of the six sites. However, variation partitioning analysis suggested that the impact of tidal changes was limited compared to other factors in both regions. These findings suggest that tidal movements may not be a critical factor in designing sampling plans for comparing fish community structures through eDNA metabarcoding at this spatial and temporal scale. Additionally, they highlight the need for empirical studies to explore the underlying mechanisms driving differences in eDNA-based fish community composition related to tidal changes.

Key words: Biodiversity monitoring, conservation, eDNA metabarcoding, fish community composition, tidal changes, tidal current

OPEN ACCESS

Academic editor: Ana Filipa Filipe
Received: 27 August 2024
Accepted: 28 November 2024
Published: 20 December 2024

Citation: Oka S-ichiro, Sado T, Ushio M, Miya M (2024) Assessing the impact of tidal changes on fish environmental DNA metabarcoding in temperate and tropical coastal regions of Japan. Metabarcoding and Metagenomics 8: e135461. https://doi.org/10.3897/mbmg.8.135461

Introduction

Environmental DNA (eDNA) metabarcoding has revolutionised aquatic biodiversity studies, especially in fish community monitoring, by providing a rapid, cost-effective and non-invasive method (Taberlet et al. 2012; Kelly et al. 2014; Deiner et al. 2017; Sigsgaard et al. 2017; Ruppert et al. 2019; Miya 2022). This technique allows for the simultaneous detection and identification of multiple species through eDNA, effectively addressing the challenges associated with

traditional observation methods (e.g. diving) and direct sampling (e.g. netting) (Taberlet et al. 2012; Miya 2022). The growing application of eDNA metabarcoding in ecological research and conservation biology underscores its effectiveness in revealing species diversity and distribution across both marine and freshwater environments (Ahn et al. 2020; Beng and Corlett 2020; Miya 2022).

In eDNA studies, the water sampling process is essential for accurately capturing biodiversity in various ecosystems (Beng and Corlett 2020; Miya 2022; Alexander et al. 2023; Patin and Goodwin 2023). The accuracy and fidelity of eDNA samples are heavily influenced by the sampling strategy, which includes factors such as location, depth and timing of collection (Kelly et al. 2018; Alexander et al. 2023; Dan et al. 2024). These aspects are crucial as they significantly affect the eDNA's concentration and composition within the samples. In marine environments, factors like tidal currents and wave action might impact eDNA distribution (Kelly et al. 2018; Sevellec et al. 2024), whereas in rivers and lakes, flow patterns and water turnover rates are important considerations (Jane et al. 2015; Stoeckle et al. 2017; Qian et al. 2023). Thorough and strategic water sampling ensures a more faithful representation of the biodiversity present, thus enhancing the reliability and interpretability of eDNA metabarcoding results in diverse aquatic environments (Miya et al. 2020; Miya 2022; Cote et al. 2023).

Understanding the dynamics of eDNA in response to tidal changes is crucial due to its significant impact on the transport and dispersal of eDNA in coastal regions (Kelly et al. 2018; Lafferty et al. 2021; Sevellec et al. 2024). During incoming tides, water masses flow towards the coast from offshore, potentially transporting eDNA released by organisms in adjacent habitats. Conversely, outgoing tides may result in water moving away from the coast, influencing eDNA accumulation or dispersal (Kelly et al. 2018; Sevellec et al. 2024). These tidal-induced fluctuations in water flow can significantly alter both the quantity and quality of eDNA in the environment. Therefore, analysing the spatiotemporal dynamics of eDNA in the intertidal zone, where water influx and efflux are most pronounced, is important for accurately assessing species presence and understanding community dynamics.

Despite its potential impact on eDNA dynamics, research on the tidal influences remains limited. For example, Kelly et al. (2018) investigated the impact of tidal changes on coastal marine organisms in high-latitude fjords and found that nearshore organismal communities were largely consistent across tides. Lafferty et al. (2021) noted that tides did not disrupt eDNA signals from sandflat fish communities. Sevellec et al. (2024) tracked the dynamics of eDNA in response to tidal changes in estuarine and coastal regions of high-latitude areas. Their findings revealed that the community structure in estuaries exhibits significant fluctuations with tidal changes, whereas coastal communities remain more stable in response to such variations. Comprehensive investigations in diverse, species-rich environments, particularly in lower latitudes, is critical to understanding how tidal changes affect eDNA. Improved insight into these relationships is key for the accurate interpretation of eDNA data, which is essential for effective conservation and ecological management in marine ecosystems.

The primary aim of this study was to investigate the impact of tidal fluctuations on the composition of fish communities as detected through eDNA, with a particular focus on coastal areas with pronounced tidal variations. To this end, we selected two coastal regions in Japan, located at 35°N and 25°N, known for

their temperate and tropical fish faunas, respectively. Sampling was strategically timed to coincide with spring tides, characterised by the most significant tidal height differences (approximately 1.5–2 m). Over a span of two days, water samples were systematically collected during both outgoing and incoming tides, resulting in four distinct sampling events. In each region, samples were collected from three sites within a 20-km radius, with three replicates at each site, totalling 72 samples. These samples were then subjected to fish eDNA metabarcoding analysis. We expected that this data-driven approach would bridge the gaps in our understanding of the relationships between tidal fluctuations and the compositions of detected fish communities.

Methods

Ethics statement

The field survey had been conducted in compliance with Japanese and local laws and regulations.

Water sampling and filtration

Seawater samples were collected over two consecutive days (16, 17 May 2022) from three sites each on the southern tip of the Boso Peninsula along the Pacific coast of temperate Japan (Fig. 1A, B) and the western tip of the Motobu Peninsula on Okinawa-jima Island, facing the East China Sea in tropical Japan (Fig. 1A, C). Hereafter, the two Peninsulas are referred to as "regions," whereas sampling sites in each region are referred to as "sites." The selected sites, all within a 20 km radius in each region, facilitated efficient sampling within a short timeframe. The Boso Peninsula sites (BP1–3) had rocky shores (Fig. 1B), while Motobu Peninsula (MP1–3) were situated in a coral reef lagoon (Fig. 1C).

At each site, four rounds of seawater sampling were conducted during both outgoing and incoming tides, exclusively in daylight (Fig. 1D). Before collecting seawater, disposable gloves were worn on both hands. A simple bucket method was employed, where surface seawater was collected using sterilised bucket. The seawater was then transferred into a sterile plastic bag (DP16-TN1000, COWPACK LTD), with three 1000 ml replicate samples per event (12 samples per site). To prevent eDNA degradation (Yamanaka et al. 2017), 1 ml of benzalkonium chloride was added immediately after sampling and the bags were sealed. Water temperature and salinity were measured on-site with a portable meter. The samples were transported to our facilities in a cooling box with ice packs. Collection details are summarised in Suppl. material 1.

Water samples were filtered on the day of sampling using a gravity filtration system (Oka et al. 2022) equipped with Sterivex filter cartridges (nominal pore size = 0.45 µm; Merck Millipore). After filtration, the outlet port of the cartridges was sealed with rubber caps (KOKUGO, Tokyo, Japan) and 1.6 ml of RNAlater (Thermo Fisher Scientific, DE, USA) was added using a disposable capillary pipette (AS ONE, Tokyo, Japan) to prevent eDNA degradation. The inlet port was then sealed with a screw cap (TERUMO, Tokyo, Japan). Additionally, filtration blank (FB) was prepared by filtering 1,000 ml of Milli-Q water in the same manner as the seawater samples.

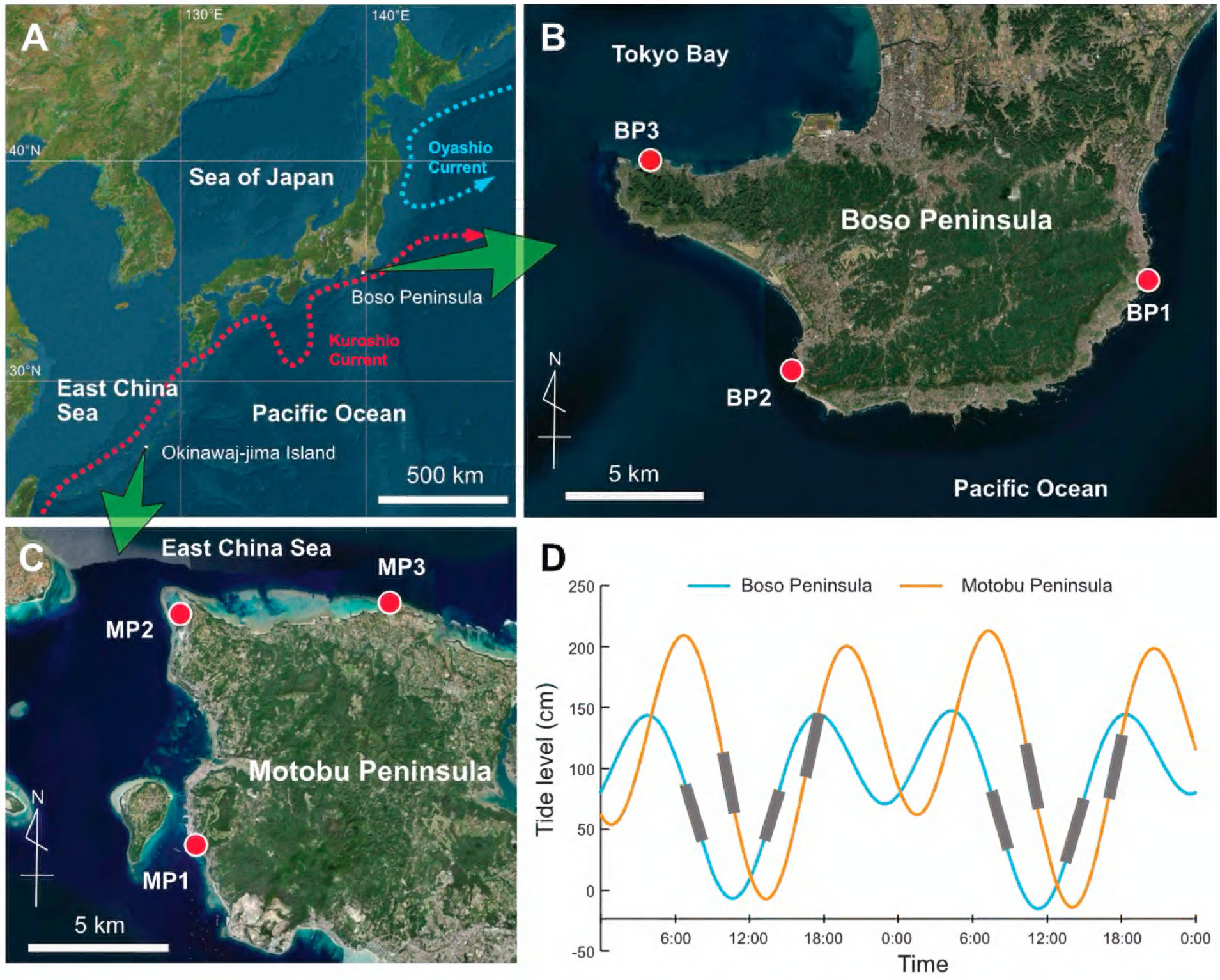

Figure 1. **A** map showing schematic flow paths of the Kuroshio Current (red) and Oyashio Current (blue), along with the geographic locations of the two Peninsulas **B** water sampling sites on the Boso Peninsula **C** on the Motobu Peninsula (map data: 2021 Apple Inc) **D** tidal changes in the two sampling regions with the timing of sampling indicated by bold lines.

Laboratory protocols

The workspace and equipment were thoroughly sterilised before laboratory experiments. Filtered pipette tips were used and eDNA extraction, as well as pre- and post-PCR manipulations, were performed in three physically separated rooms to minimise possible cross-contamination (Miya et al. 2020).

eDNA was extracted using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), following Miya et al. (2016) with slight modifications. Initially, the preservative in the cartridge was removed and then the filter membrane was lysed using proteinase K. The resulting DNA extract, approximately 500 µl, was purified using the same kit according to the manufacturer's protocol, with the final elution volume adjusted to 200 µl. An extraction blank (EB) was also prepared concurrently.

A two-step PCR was employed for paired-end library preparation using the MiSeq platform (Illumina, CA, USA), following methods adapted from Miya et al. (2015) and Miya and Sado (2019). In the first round (1st PCR), a mixture of three primer pairs (Mifish-U, Mifish-E-v2 and Mifish U2) was used in a 0.9:1.0:0.1 ratio. These primers target a hypervariable region of the mitochondrial 12S rRNA gene (approximately 172 bp, referred to as the "MiFish sequence") and to

append primer-binding sites (at the 5´ ends of the sequences before six Ns) for sequencing at both ends of the amplicon.

The 1st PCR included eight technical replicates per eDNA template to minimise PCR dropouts (Doi et al. 2019). Additionally, a 1st PCR blank (1B) was prepared, alongside the field and extraction blanks (FB and EB). After amplification, an equal volume of PCR products from each of the replicates was pooled, purified, quantified and diluted to 0.1 ng/µl using Milli Q water. These diluted products were used as templates for the second round (2nd PCR), processed in an identical manner for the blanks.

The 2nd PCR was conducted to append dual-index sequences and flow cell binding sites necessary for the MiSeq platform. Alongside the eDNA samples, a 2nd PCR blank was prepared, in addition to FB, EB and 1B. The PCR products from the 2nd PCR, as well as the four blank samples (FB, EB, 1B and 2B), were pooled with other samples from different projects. Subsequently, the pooled dual-indexed libraries were subjected to electrophoresis on an agarose gel. The target amplicons (approximately 370 bp) were then excised from the gel. The concentration of the size-selected libraries was measured and diluted to 10.0 pM. Finally, sequencing was performed on the MiSeq platform using a MiSeq v.2 Reagent Kit for 2 × 150 bp PE (Illumina, CA, USA), according to the manufacturer's protocol.

All raw DNA sequence data and associated information have been deposited in the DDBJ/EMBL/GenBank databases. The sequence data are available from these databases with the accession numbers DRA016351 and DRA016352.

Data preprocessing and taxon assignment

Data preprocessing and analysis of raw MiSeq reads were performed using PMiFish ver. 2.4 (https://github.com/rogotoh/PMiFish.git; Miya et al. (2020)). Forward (R1) and reverse (R2) reads were merged, while discarding low-quality tail reads with a cut-off threshold at a quality (Phred) score of 2. Reads shorter than 100 bp post-tail trimming and paired reads with excessive mismatches (> 5 positions) in the aligned region (approximately 65 bp) were excluded from further analysis. Primer sequences were removed from the merged reads and those lacking primer sequences underwent further quality filtering to eliminate low-quality reads. The preprocessed reads were then dereplicated, removing all singletons, doubletons and tripletons from subsequent analyses to reduce false positives (Edgar 2010). The dereplicated reads were denoised to generate amplicon sequence variants (ASVs), thereby removing all sequences deemed putatively chimeric or erroneous (Callahan et al. 2017).

The ASVs were assigned to species names (molecular operational taxonomic units; MOTUs) with a sequence identity of > 98.5% with the reference sequences (allowing for two nucleotide differences) and a query coverage of ≥ 90%. This clustering step, necessitated by an incomplete reference database, enables the detection of multiple MOTUs for identical species names with sequence identities of < 98.5%. These were annotated as "gotu1, 2, 3..." and all outputs (MOTUs plus U98.5 MOTUs) were tabulated with read abundances. ASVs with sequence identities of < 80% (saved as "no hit") were excluded from taxon assignments and downstream analyses, as they were identified as non-fish organisms. MiFish DB ver. 43 was used, encompassing 8,523 species across 489 families and 2,808 genera.

To refine the taxon assignments, family-level phylogenies were reconstructed using MiFish sequences from MOTUs and reference sequences from the corresponding families in MiFish DB ver. 43. Representative sequences (those with the highest reads abundances) for each family were combined with the reference sequences and saved in FASTA format. These sequences were then aligned using MAFFT 7 (Katoh and Standley 2013) with the default parameters. The aligned sequences were used to construct a Neighbour-joining (NJ) tree in MEGA X (Stecher et al. 2020), applying Kimura two-parameter distances.

All family-level trees were visually inspected and taxon assignments were revised as follows: U98.5 MOTUs within a monophyletic group of a single genus were named after that genus, followed by "sp." and sequential numbers (e.g. *Pagrus* sp. 1, sp. 2, sp. 3...). For MOTUs ambiguously placed in the family-level tree, the respective family name was used, followed by "sp." with sequential numbers (e.g. Sparidae sp. 1, sp. 2, sp. 3...).

Statistical analysis

Fish community structures were analysed using non-metric multidimensional scaling (NMDS), based on incidence-based Jaccard indices. The stress value was used to confirm the representation of NMDS ordination.

Variation in fish communities explained by sampling sites (n = 3 per peninsula), tidal phases (outgoing vs. incoming) and sampling dates (n = 2 per sampling site) was analysed using variation partitioning (Peres-Neto et al. 2006). The "date" variable was included to assess its relative importance compared to tidal phases. Both NMDS and variation partitioning utilised incidence-based Jaccard indices were implemented using "vegan" package in R (Oksanen et al. 2022). The R code used for the variation partitioning analysis is provided in the Suppl. material 7.

Indicator taxa analysis (Cáceres and Legendre 2009) was conducted to identify taxa with significantly different detection frequencies between outgoing and incoming tides at each site.

All statistical analyses were conducted under R v.4.2.2 (R Core Team 2022) with the following packages: iNEXT (v.3.0.0; Hsieh et al. (2016)), vegan (v.2.6–2; Oksanen et al. (2022)), VennDiagram (v.1.7.3; Chen (2022)) and indicspecies (v.1.7.13; Cáceres (2023)).

Results and discussion

Overview of the survey

The survey period (16, 17 May 2022) coincided with the spring tide phase, featuring daily tidal differences of approximately 1.5–2 metres (Fig. 1D). Over the two consecutive days, four rounds of seawater sampling were completed at three sites on each Peninsula, with each round taking about an hour (Suppl. material 1). The weather alternated between light rain and cloudiness, with no significant wind. Calm weather in the preceding days resulted in exceptionally clear seawater in both regions, reducing potential disturbances to fish communities and limiting the advection of eDNA adjacent habitat. These stable conditions minimised confounding factors in our eDNA analysis.

Sequencing performance and data preprocessing

Two MiSeq paired-end sequencing runs (2 × 150 bp) were performed for the 80 libraries including eight blanks (two sets of FB, EB, 1B and 2B), yielding a total of 8,594,144 reads. On average, 96.6% of base calls reached Phred quality scores of ≥ 30.0 (Q30; a base call accuracy of 99.9%). This sequencing performance significantly exceeds Illumina's guideline of > 80% bases at ≥ Q30 for 2 × 150 bp, as specified in Illumina Publication No. 770-2011-001 as of 27 May 2014.

These reads were assigned to the 80 libraries, including the eight blanks. The raw read counts for the 72 non-blank libraries were relatively uniform, ranging from 61,951 to 178,198 with a mean of 119,363 reads. After merging the two overlapping paired-end fastq files, resulting in 8,445,044 reads (98.3% of the initial reads), the primer-trimmed sequences underwent quality filtering to eliminate low-quality reads, leaving 8,373,035 reads (97.4%). These reads were then dereplicated for further analysis, with singletons to tripletons excluded from the unique sequences. Subsequent denoising removed putatively erroneous and chimeric sequences, leaving 7,335,844 reads (85.4%) to be subjected to taxon assignments.

The eight blank libraries, processed identically to the non-blank libraries, yielded no reads after denoising, confirming effective contamination control and enhancing the reliability of sequencing data from the 72 samples.

Taxonomic assignment

Of the 7,335,844 denoised reads, 7,299,867 (99.5%) were tentatively identified as fish sequences based on ≥ 80% sequence identity with reference data from MiFish DB ver. 43. BLAST searches of sequences with < 80% identity (35,977 reads or 0.49%) indicated they were primarily from terrestrial mammals (e.g. cows, pigs and humans) or unidentified sequences. Following automatic taxon assignments, species presumed to be of deep-sea or purely freshwater origin (29 detections and 9,608 reads) were excluded from the list (Suppl. material 2), narrowing our focus to the detection of coastal fish at the six target sites along the two Peninsulas. The presence of these ostensibly exogenous eDNAs from adjacent ecosystems was further analysed to assess the impact of tidal movements on potential false positive detections (see below).

After excluding exogenous sequences, family-level NJ (Neighbour-joining) phylogenetic trees were visually inspected to refine species assignment. This process identified four putatively chimeric sequences (18 reads total) from four detections, which were also excluded. The final dataset included 4,446 detections assigned to 595 species, 92 families and 295 genera (Suppl. material 3).

Between-region species diversity

In the Boso Peninsula, species richness per sample ranged from 19 to 66 with a mean of 40.4, whereas in the Motobu Peninsula, it ranged from 34 to 154 with a mean of 83.1 (Fig. 2A), reflecting typical latitudinal diversity patterns of fish communities (Mora et al. 2003; Bosch et al. 2021). A total of 152 species were identified in the Boso Peninsula and 475 in the Motobu Peninsula (Fig. 2B, Suppl. material 3). Amongst the 595 species identified overall, only

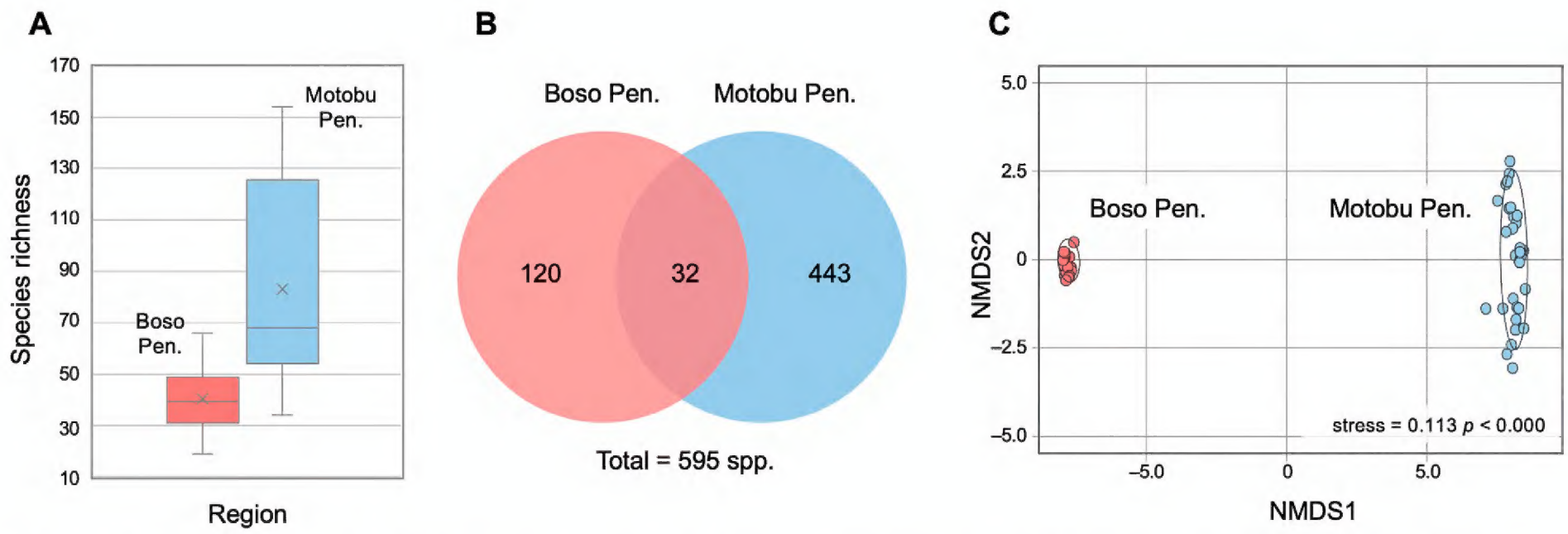

Figure 2. A box plot showing variations in species richness across the two Peninsulas **B** venn diagram showing unique and shared species between the two Peninsulas **C** NMDS ordination analysis of fish community compositions between the two Peninsulas.

32 were common to both Peninsulas (Fig. 2B), including *Kyphosus bigibbus* (Kyphosidae), *Atherion elymus* (Atherionidae, and *Mugil cephalus cephalus* (Mugilidae) (Suppl. material 3), which are widely distributed along the Kuroshio Current region (Nakabo 2013). Non-metric multidimensional scaling (NMDS) ordination analysis clearly highlighted the significant differences in fish community compositions between the two regions (Fig. 2C).

Regarding frequently detected species, each Peninsula showed a distinct set of species consistently found across samples, aligned with known distribution data (Nakabo 2013). In the Boso Peninsula, six temperate species, associated with reef or sandy-bottom habitats, were detected in all 36 samples: *Acanthopagrus schlegelii* (Sparidae), *Goniistius zonatus* (Cheilodactylidae), *Thalassoma cupido* (Labridae), *Enneapterygius etheostomus* (Tripterygiidae), *Istiblennius enosimae* (Blenniidae) and *Takifugu alboplumbeus* (Tetraodontidae) (Suppl. material 3). In contrast, in the Motobu Peninsula, no single species was found in all the samples. The six most frequently detected species, such as *Siganus spinus* (Siganidae), *Chrysiptera cyanea* (Pomacentridae), *Nectamia fusca* (Apogonidae), *Ctenochaetus striatus* (Acanthuridae), *Gerres oyena* (Gerreidae) and *Acanthurus nigrofuscus* (Acanthridae), associated with coral reefs, were present in 28 to 34 samples, indicating a clear ecological distinction between the two regions.

Between-site species diversity

Based on the observed distinctions in between-region species diversity (Fig. 2A–C), we further investigated the variations in species richness and fish community compositions between sites within each Peninsula by employing a consistent analytical framework (Fig. 3A–F).

When comparing species richness amongst sites within each Peninsula, we detected a range of 67 to 120 species on the Boso Peninsula and 219 to 324 species on the Motobu Peninsula across three respective sites (Fig. 3A, D). Analysis of the variability in detected species per sample revealed statistically significant differences for both peninsulas ($p < 0.0001$; Kruskal-Wallis test). However, no significant difference was observed between the MP1/MP2 pair ($p = 0.684$; Dunn's multiple comparison test).

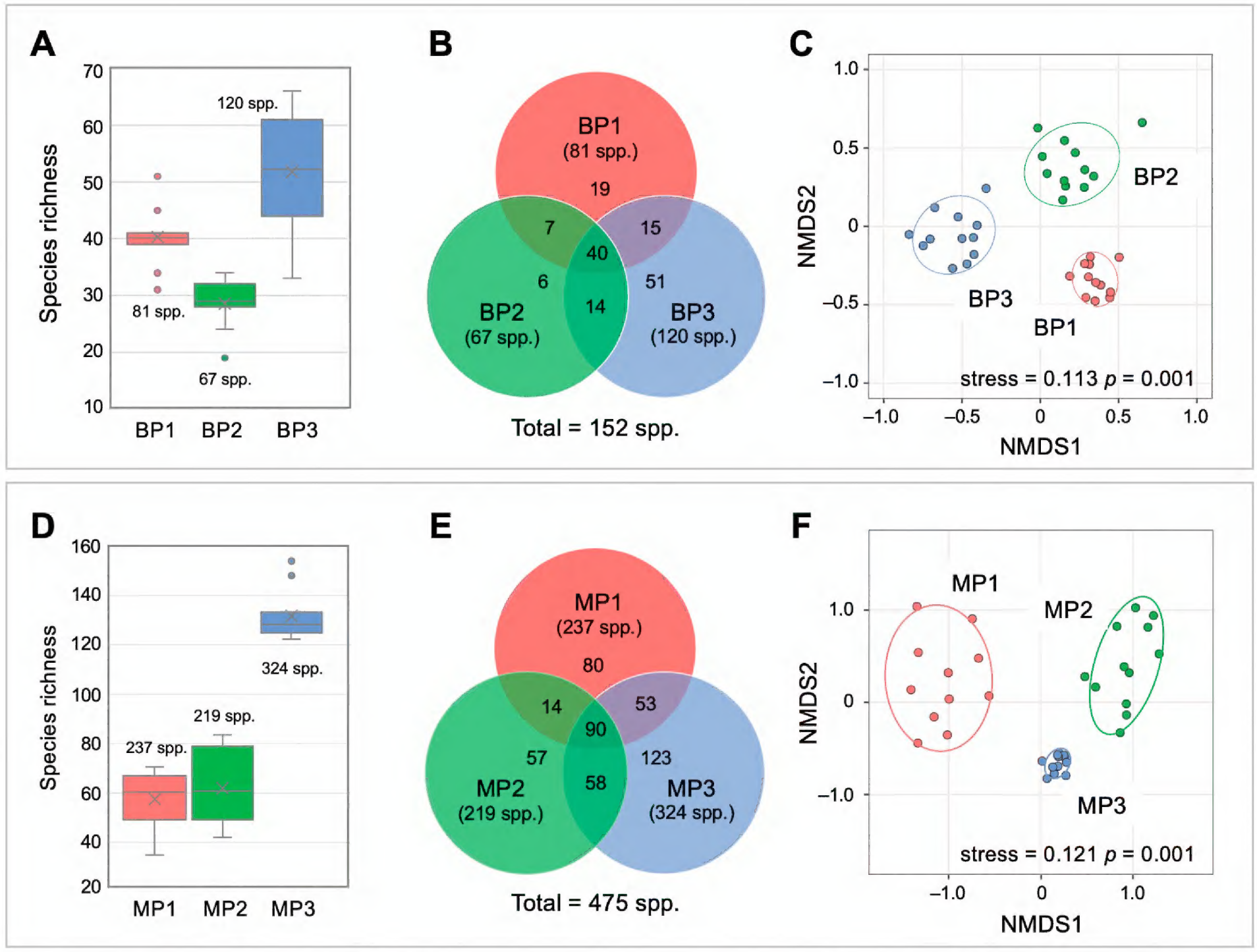

Figure 3. A, D box plots showing variations in species richness across the three sites on the Boso Peninsula and the three sites on the Motobu Peninsula, respectively **B, E** venn diagrams showing unique and shared species amongst the three sites on each Peninsula **C, F** NMDS ordination analysis of fish community compositions amongst the three sites on each Peninsula.

Upon examining the detected species overlap amongst the three sites (Fig. 3B, E), we noted that the lowest total (BP = 67 and MP = 219) and uniquely detected species counts (BP = 6 and MP = 57) were both recorded at the intermediate sites (BP2 and MP2). Additionally, BP2 and MP2 exhibited the highest proportion of commonly detected species with the other two sites (BP: 91.0% vs. 76.5 and 57.5%; MP: 74.0% vs. 66.2 and 62.0%), highlighting the significant influence of relative site position on fish community composition (Sigsgaard et al. 2017; Kume et al. 2021; Stoeckle et al. 2024).

NMDS ordination analysis of the differences in fish community composition clearly distinguished the fish communities at the three sites in a two-dimensional space (Fig. 3C, F; $p = 0.001$). Moreover, when comparing the variability in the dissimilarity index across all combinations of fish community compositions at three levels — within sites, between adjacent sites and between distant sites — the values within sites were smaller on both Peninsulas, with a noticeable trend towards larger dissimilarities between adjacent or distant sites (Fig. 4).

The two adjacent sites on each Peninsula are located within 10 to 15 km of each other along the coast. Despite this proximity, significant variations in species richness and fish community compositions are observed amongst

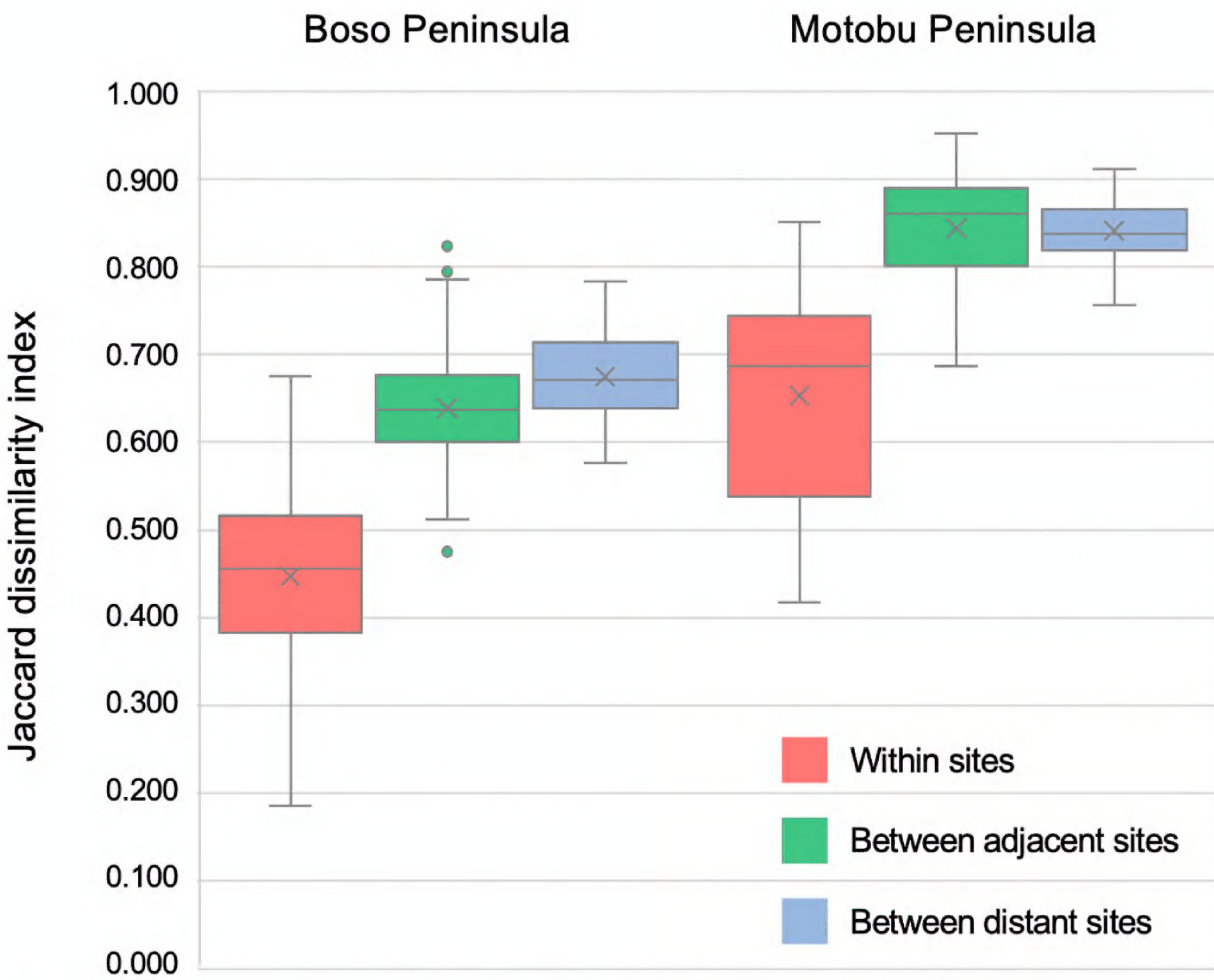

Figure 4. Box plots showing variations in species richness within sites, between adjacent sites and between distant sites on the Boso and Motobu Peninsulas.

some of the sites on each Peninsula, attributable to their distinct environmental characteristics.

The Kuroshio Current, flowing approximately 50 km northwards off the Boso Peninsula (Fig. 1A), significantly influences all three sites. However, the notably higher species richness (Fig. 3A) and resulting higher number of unique species at BP3 (Fig. 3B) may be attributed to its location in Tateyama Bay, where an offshoot of the current flows directly in (Hagiwara and Kimura 2006). Consequently, coral colonies — recorded as the northernmost in Japanese coastal waters — are scattered throughout the area, facilitating year-round observation of tropical fish species (Miya 2022). In fact, at BP3, tropical fish species, such as *Parascorpaena mossambica* (Scorpaenidae), *Epinephelus areolatus* (Serranidae) and *Pomacentrus coelestis* (Pomacentridae), were exclusively detected.

Similarly, on the Motobu Peninsula, MP3 exhibits remarkably higher species richness (Fig. 3D) and the resultant higher number of unique species (Fig. 3E). This site, located within a lagoon like the other two sites (MP1/2), is characterised by a shorter distance from the shore to the reef edge (Fig. 1C). The difference in fish community composition between the reef edge and the shore-side area within the lagoon at MP2, as reported by Oka et al. (2021), might have contributed to the increased species diversity at MP3, where both habitats are closely located.

Tidal impact on species diversity

Given the observed distinctions in both between-region and between-site species diversity (Figs 2–4), we further explored the impacts of tidal changes (outgoing vs. incoming tides) on the variations in species richness and the composition of fish communities at individual sites using a consistent analytical framework (Fig. 5).

We plotted the variability in the number of detected species at each site across the two tidal phases (Fig. 5A, D). Despite some overlap in variability across sites, the Wilcoxon signed-rank test revealed statistically significant differences at two specific sites (BP3 and MP3; p = 0.035), though the differences were marginally significant. When examining the species overlap between the two tidal phases, we consistently found that the number of species common to both outgoing and incoming tides was higher at every site, ranging from 43 to 186. In contrast, species unique to a specific tidal phase varied from 9 to 80 (Fig. 5B, E). In terms of unique species proportions, the Boso Peninsula showed a lower range (33.3–37.5%) compared to the Motobu Peninsula (42.6–58.4%).

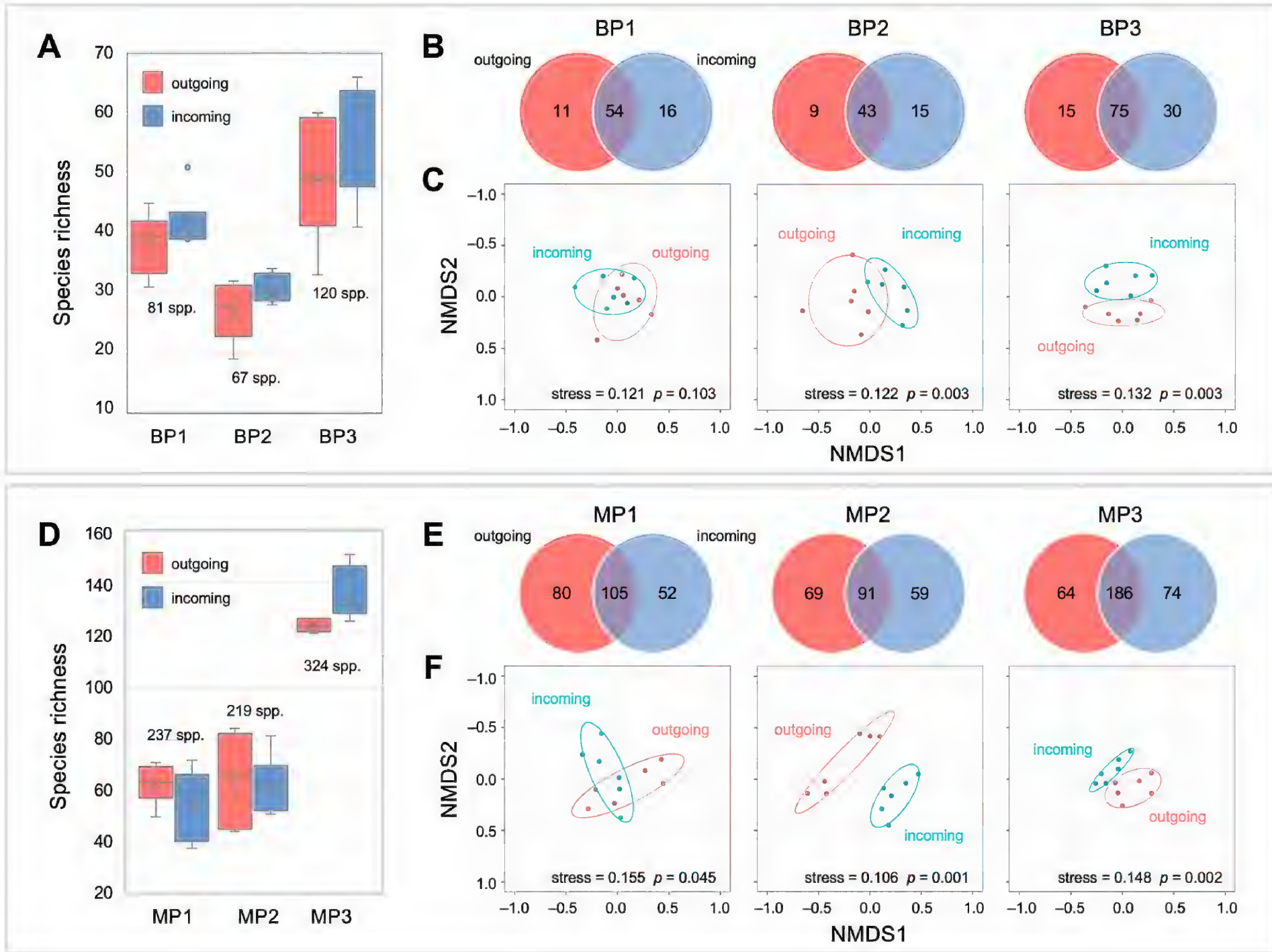

Figure 5. **A, D** box plots showing variations in species richness between outgoing and incoming tides across the three sites on the Boso and Motobu Peninsulas, respectively **B, E** venn diagrams showing unique and shared species between outgoing and incoming tides across the three sites on each Peninsula **C, F** NMDS ordination analysis of fish community compositions between outgoing and incoming tides across the three sites on each Peninsula.

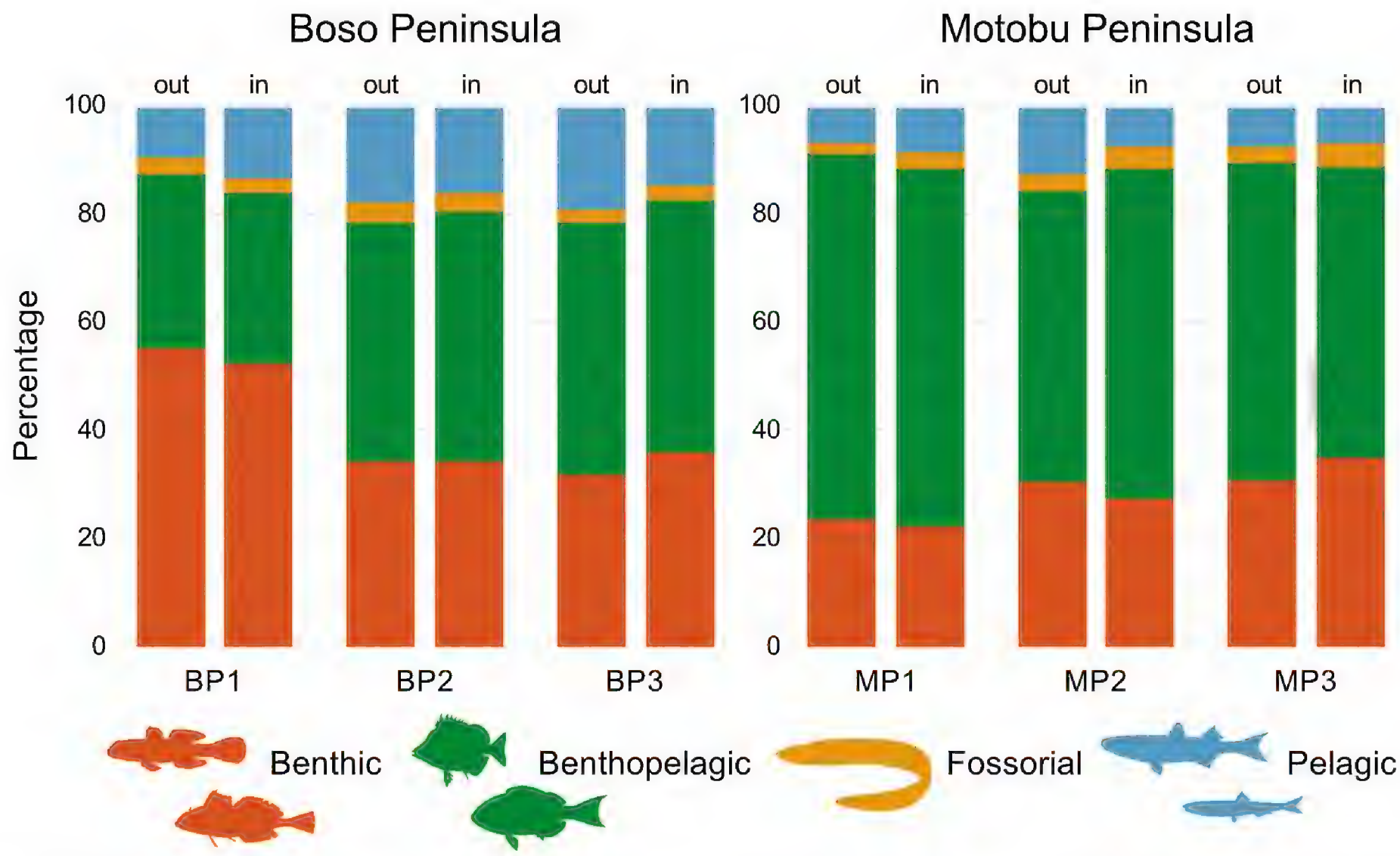

Figure 6. Bar charts showing the proportions of four ecological categories of fish species, comparing outgoing and incoming tides across the three sites on each Peninsula.

NMDS ordination revealed significant differences in fish community compositions between tidal phases at five of the six sites, except BP1 (Fig. 5C, F; $p < 0.05$). Indicator species analysis identified 10 species linked to specific tidal phases: five each for outgoing tide and incoming tides, primarily from Motobu Peninsula (Suppl. material 4). The limited indicator species on the Boso Peninsula likely reflects fewer unique species per tidal phase. These species were common across the Peninsulas, showing no distinct ecological patterns (Hagiwara and Kimura 2006; Nakabo 2013).

Beyond community composition, we classified the detected fish species into four ecological categories: benthic, benthopelagic, fossorial and pelagic. We then examined their proportional representation during the outgoing and incoming tides (Fig. 6). A consistent pattern emerged across both Peninsulas, with benthic and benthopelagic species together comprising over 70% of the total. No statistically significant differences in the distribution of these ecological types were detected between the two tidal phases at any site (chi-squared test, $p > 0.05$). On the Motobu Peninsula, the prevalence of benthopelagic species likely reflects the high number of coral reef-associated fishes, such as those from the butterflyfish (Chaetodontidae) and damselfish (Pomacentridae) (Nakabo 2013; Hobbs and Srinivasan 2024; McClanahan et al. 2024).

Variation partitioning showed that the sampling sites explained the largest variation (46% in Boso and 33% in Motobu), while tidal phases accounted for only a small fraction (2.8% and 1.5%, respectively; Fig. 7). Similar results were observed within each Peninsula (Suppl. material 5), with tidal phases explaining 3.4–12.2% of the variation. Notably, the influence of tidal phases was comparable to the “date” factor, which explained 0–14.5% of the variation across sites (Fig. 7, Suppl. material 5). These findings highlight that tidal changes have

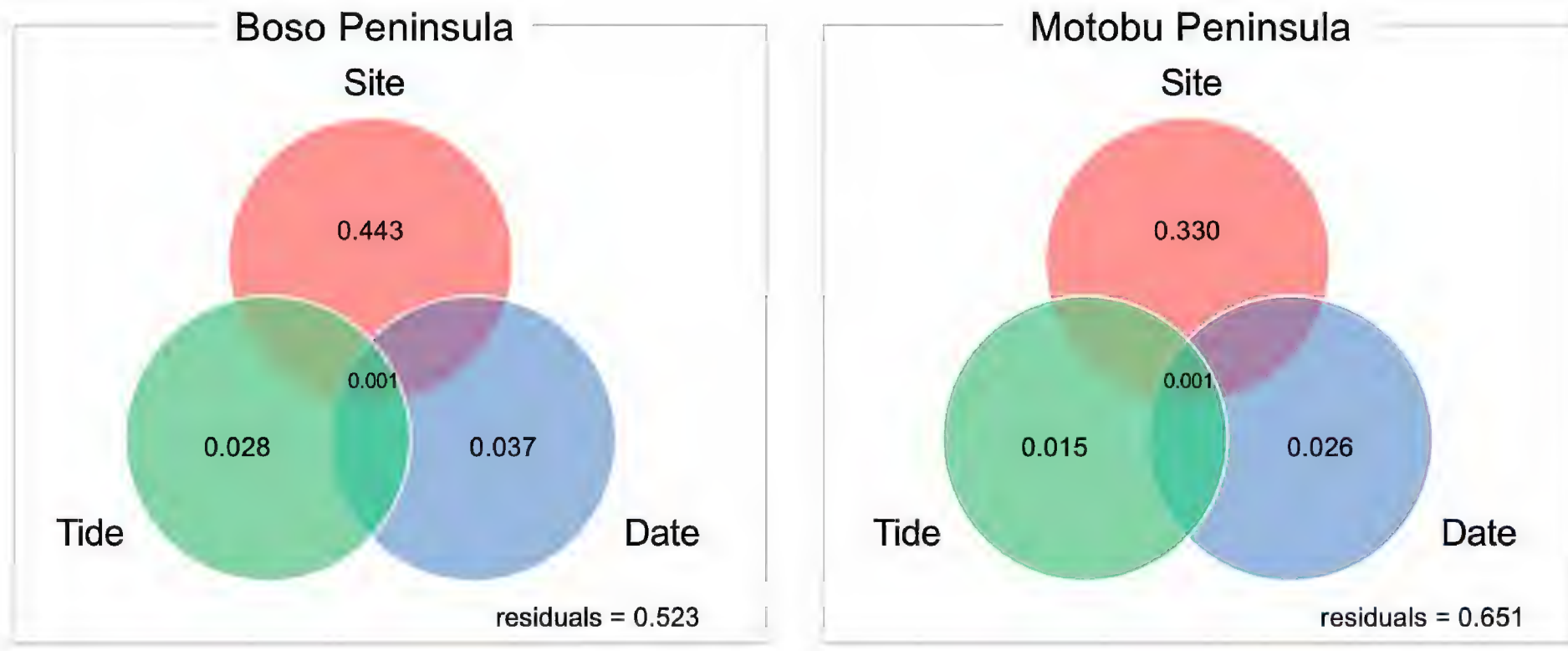

Figure 7. Venn diagrams showing the results of the variation partitioning analysis. The number in each circle indicates the proportion of explained variation by each factor. The number in each overlapping region indicates the proportion of variation simultaneously explained by more than one factor.

relatively minor impact on fish community compositions compared to site-species factors.

Comments on presumably exogenous eDNA

Although excluded from the above analysis of species diversity, 14 oceanic deep-sea fish species and six pure freshwater fish species were detected across the six sites across the two regions in this study (Suppl. material 2). The total number of these presumably exogenous DNA detections was 29, accounting for 0.65% of all detections, with 9,608 reads representing 0.13% of the total reads, both minor components at less than 1%. The weather during our survey was calm in both regions, with no rainfall and calm seas for several days prior, which might explain the seemingly low occurrence of exogenous eDNA (Cowart et al. 2022).

If this eDNA originated from adjacent ecosystems, such as oceanic deep water or freshwater areas, it is likely that freshwater fish would be detected more frequently during outgoing tides and deep-sea fish during incoming tides. In our study, five freshwater fish species with six detections were found during outgoing tides, while only one species with one detection was found during incoming tides, suggesting that influx from adjacent small rivers or sewage is a plausible explanation. Conversely, deep-sea fish showed nine species with 13 detections during outgoing tides and six species with nine detections during incoming tides, with higher species numbers and detections during outgoing tides. This pattern might suggest that the deep-sea fish eDNA originates from their larval stages when they reside in surface waters (McClenaghan et al. 2020).

Concluding remarks

In this study, we investigated the impact of outgoing and incoming tides on the detection of fish eDNA in two distinctly different regions: the temperate Boso Peninsula (35°N) and the tropical Motobu Peninsula (25°N). Over two days,

four rounds of water sampling were conducted at three sites in each region (Fig. 1), located within a 20 km radius. These sites were initially chosen as technical replicates to verify the reproducibility of the findings. Unexpectedly, it was clearly demonstrated that the three sites in each region had distinct fish communities (Fig. 3). The influence of tidal changes on the estimated composition of fish communities within each site was statistically significant in most cases (Fig. 5), but variation partitioning analysis showed that this influence was limited (Fig. 7). In summary, this suggests that, in regions with significant tidal differences, fish community surveys using eDNA can effectively capture community characteristics without the need to account heavily for tidal levels. Nevertheless, we acknowledge that significant statistical differences in fish community structures between outgoing and incoming tides were observed at five of the six sites (Fig. 5). Although the indicator species analysis did not yield clear results regarding the causes of these differences, further empirical studies on how individual fish species move in response to tidal changes and/or how eDNA is transported by the tidal flow, are necessary. On the other hand, it is important to note that even with 12 samples collected from one site over two days, species richness did not reach saturation (Suppl. material 6). Further research is needed to determine the appropriate number of samples required for a comprehensive understanding of fish communities using eDNA.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grants (Grant Numbers JP19H03291, JP22H02691, JP24K15385), the Nakatsuji Foresight Foundation Research Grant (2023) and the research budget of Okinawa Churaumi Aquarium.

Author contributions

Conceptualization: MM. Data curation: TS. Formal analysis: MM, SO, MU, TS. Funding acquisition: MM, SO. Investigation: MM, SO. Methodology: SO, TS, MU. Project administration: MM. Writing - original draft: SO, MM. Writing - review and editing: MU, TS.

Author ORCIDs

Shin-ichiro Oka https://orcid.org/0000-0002-9554-6342
Tetsuya Sado https://orcid.org/0000-0003-4149-9824
Masayuki Ushio https://orcid.org/0000-0003-4831-7181
Masaki Miya https://orcid.org/0000-0002-9791-9886

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Ahn H, Kume M, Terashima Y, Ye F, Kameyama S, Miya M, Yamashita Y, Kasai A (2020) Evaluation of fish biodiversity in estuaries using environmental DNA metabarcoding. PLoS ONE 15(10): e0231127. https://doi.org/10.1371/journal.pone.0231127

Alexander JB, Marnane MJ, McDonald JI, Lukehurst SS, Elsdon TS, Simpson T, Hinz S, Bunce M, Harvey ES (2023) Comparing environmental DNA collection methods for sampling community composition on marine infrastructure. Estuarine, Coastal and Shelf Science 283: 108283. https://doi.org/10.1016/j.ecss.2023.108283

Beng KC, Corlett RT (2020) Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. Biodiversity and Conservation 29(7): 2089–2121. https://doi.org/10.1007/s10531-020-01980-0

Bosch NE, Wernberg T, Langlois TJ, Smale DA, Moore PJ, Franco JN, Thiriet P, Feunteun E, Ribeiro C, Neves P, Freitas R, Filbee-Dexter K, Norderhaug KM, Garcıa A, Otero-Ferrer F, Espino F, Haroun R, Lazzari N, Tuya F (2021) Niche and neutral assembly mechanisms contribute to latitudinal diversity gradients in reef fishes. Journal of Biogeography 48(11): 2683–2698. https://doi.org/10.1111/jbi.14237

Cáceres MD (2023) indicspecies: Relationship between species and groups of sites. https://cran.r-project.org/web/packages/indicspecies/index.html

Cáceres MD, Legendre P (2009) Associations between species and groups of sites: Indices and statistical inference. Ecology 90(12): 3566–3574. https://doi.org/10.1890/08-1823.1

Callahan BJ, McMurdie PJ, Holmes SP (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal 11(12): 2639–2643. https://doi.org/10.1038/ismej.2017.119

Chen H (2022) VennDiagram: Generate high-resolution Venn and Euler plots. https://cran.r-project.org/web/packages/VennDiagram/index.html

Cote D, McClenaghan B, Desforges J, Fahner NA, Hajibabaei M, Chawarski J, Roul S, Singer G, Aubry C, Geoffroy M (2023) Comparing eDNA metabarcoding and conventional pelagic netting to inform biodiversity monitoring in deep ocean environments. ICES Journal of Marine Science 80: 2545–2562. https://doi.org/10.1093/icesjms/fsad169

Cowart DA, Murphy KR, Cheng C-HC (2022) Environmental DNA from Marine Waters and Substrates: Protocols for Sampling and eDNA Extraction. In: Verde C, Giordano D (Eds) Marine Genomics. Methods in Molecular Biology. Springer US, New York, NY, 225–251. https://doi.org/10.1007/978-1-0716-2313-8_11

Dan ME, Portner EJ, Bowman JS, Semmens BX, Owens SM, Greenwald SM, Choy CA (2024) Using low volume eDNA methods to sample pelagic marine animal assemblages. PLoS ONE 19: e0303263. https://doi.org/10.1371/journal.pone.0303263

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, Vere N, Pfrender ME, Bernatchez L (2017) Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular Ecology 26(21): 5872–5895. https://doi.org/10.1111/mec.14350

Doi H, Fukaya K, Oka S, Sato K, Kondoh M, Miya M (2019) Evaluation of detection probabilities at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a multispecies site occupancy model. Scientific Reports 9(1): 3581. https://doi.org/10.1038/s41598-019-40233-1

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics (Oxford, England) 26(19): 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Hagiwara K, Kimura K (2006) Fish Fauna of Shore Waters in Hasama, Tateyama, Boso Peninsula. Memoirs of the National Museum of Nature and Science 41: 351–387.

Hobbs J-PA, Srinivasan M (2024) Structure of Reef Fish Families (Butterflyfishes and Angelfishes) at Isolated Oceanic Reefs in the Indian Ocean: Christmas Island and the Cocos (Keeling) Islands. Diversity 16(9): 569. https://doi.org/10.3390/d16090569

Hsieh TC, Ma KH, Chao A (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods in Ecology and Evolution 7: 1451–1456. https://doi.org/10.1111/2041-210X.12613

Jane SF, Wilcox TM, McKelvey KS, Young MK, Schwartz MK, Lowe WH, Letcher BH, Whiteley AR (2015) Distance, flow and PCR inhibition: E DNA dynamics in two headwater streams. Molecular Ecology Resources 15(1): 216–227. https://doi.org/10.1111/1755-0998.12285

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kelly RP, Port JA, Yamahara KM, Crowder LB (2014) Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE 9: e86175. https://doi.org/10.1371/journal.pone.0086175

Kelly RP, Gallego R, Jacobs-Palmer E (2018) The effect of tides on nearshore environmental DNA. PeerJ 6: e4521. https://doi.org/10.7717/peerj.4521

Kume M, Lavergne E, Ahn H, Terashima Y, Kadowaki K, Ye F, Kameyama S, Kai Y, Henmi Y, Yamashita Y, Kasai A (2021) Factors structuring estuarine and coastal fish communities across Japan using environmental DNA metabarcoding. Ecological Indicators 121: 107216. https://doi.org/10.1016/j.ecolind.2020.107216

Lafferty KD, Garcia-Vedrenne AE, McLaughlin JP, Childress JN, Morse MF, Jerde CL (2021) At Palmyra Atoll, the fish-community environmental DNA signal changes across habitats but not with tides. Journal of Fish Biology 98(2): 415–425. https://doi.org/10.1111/jfb.14403

McClanahan T, Friedlander A, Chabanet P, Bruggemann J, Wickel J, Azali M (2024) Modeling the spatial distribution of numbers of coral reef fish species and community types in the Western Indian Ocean faunal province. Marine Ecology Progress Series 730: 59–78. https://doi.org/10.3354/meps14538

McClenaghan B, Fahner N, Cote D, Chawarski J, McCarthy A, Rajabi H, Singer G, Hajibabaei M (2020) Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. PLoS ONE 15: e0236540. https://doi.org/10.1371/journal.pone.0236540

Miya M (2022) Environmental DNA metabarcoding: A novel method for biodiversity monitoring of marine fish communities. Annual Review of Marine Science 14: 161–185. https://doi.org/10.1146/annurev-marine-041421-082251

Miya M, Sado T (2019) Multiple species detection using MiFish primers. In: eDNA Method standardization committee (Eds) Environmental DNA Sampling and Experiment Manual Version 2.1. The eDNA Society, Otsu, Japan, 55–92.

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Minamoto T, Yamamoto S, Yamanaka H, Araki H, Kondoh M, Iwasaki W (2015) MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. Royal Society Open Science 2(7): 150088. https://doi.org/10.1098/rsos.150088

Miya M, Minamoto T, Yamanaka H, Oka S, Sato K, Yamamoto S, Sado T, Doi H (2016) Use of a filter cartridge for filtration of water samples and extraction of environmental DNA. Journal of Visualized Experiments 117: e54741. https://doi.org/10.3791/54741-v

Miya M, Gotoh RO, Sado T (2020) MiFish metabarcoding: A high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. Fisheries Science 86(6): 939–970. https://doi.org/10.1007/s12562-020-01461-x

Mora C, Chittaro PM, Sale PF, Kritzer JP, Ludsin SA (2003) Patterns and processes in reef fish diversity. Nature 421(6926): 933–936. https://doi.org/10.1038/nature01393

Nakabo T [Ed.] (2013) Fishes of Japan with pictorial keys to the species 3rd edn. Tokai University Press, Kanagawa-ken Hadano-shi, 2440 pp.

Oka S, Doi H, Miyamoto K, Hanahara N, Sado T, Miya M (2021) Environmental DNA metabarcoding for biodiversity monitoring of a highly diverse tropical fish community in a coral reef lagoon: Estimation of species richness and detection of habitat segregation. Environmental DNA 3(1): 55–69. https://doi.org/10.1002/edn3.132

Oka S, Miya M, Sado T (2022) Gravity filtration of environmental DNA: A simple, fast, and power-free method. MethodsX 9: 101838. https://doi.org/10.1016/j.mex.2022.101838

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, McBolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista HBAE (2022) Package "vegan": Community Ecology Package. https://cran.r-project.org/web/packages/vegan/vegan.pdf

Patin NV, Goodwin KD (2023) Capturing marine microbiomes and environmental DNA: A field sampling guide. Frontiers in Microbiology 13: 1026596. https://doi.org/10.3389/fmicb.2022.1026596

Peres-Neto PR, Legendre P, Dray S, Borcard D (2006) Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology 87(10): 2614–2625. https://doi.org/10.1890/0012-9658(2006)87[2614:VPOSDM]2.0.CO;2

Qian M-M, Wang Z-Y, Zhou Q, Wang J, Shao Y, Qiao Q, Fan J-T, Yan Z-G (2023) Environmental DNA unveiling the fish community structure and diversity features in the Yangtze River basin. Environmental Research 239: 117198. https://doi.org/10.1016/j.envres.2023.117198

R Core Team (2022) R: A language and environment for statistical computing. https://www.r-project.org

Ruppert KM, Kline RJ, Rahman MS (2019) Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation 17: e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sevellec M, Lacoursière-Roussel A, Normandeau E, Bernatchez L, Howland K (2024) Tidal effect on environmental DNA communities in Arctic estuarine and marine ecosystems. Frontiers in Marine Science 11: 1390489. https://doi.org/10.3389/fmars.2024.1390489

Sigsgaard EE, Nielsen IB, Carl H, Krag MA, Knudsen SW, Xing Y, Holm-Hansen TH, Møller PR, Thomsen PF (2017) Seawater environmental DNA reflects seasonality of a coastal fish community. Marine Biology 164(6): 128. https://doi.org/10.1007/s00227-017-3147-4

Stecher G, Tamura K, Kumar S (2020) Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Molecular Biology and Evolution 37: 1237–1239. https://doi.org/10.1093/molbev/msz312

Stoeckle BC, Beggel S, Cerwenka AF, Motivans E, Kuehn R, Geist J (2017) A systematic approach to evaluate the influence of environmental conditions on eDNA detection success in aquatic ecosystems. PLoS ONE 12: e0189119. https://doi.org/10.1371/journal.pone.0189119

Stoeckle MY, Ausubel JH, Hinks G, VanMorter SM (2024) A potential tool for marine biogeography: eDNA-dominant fish species differ among coastal habitats and by season concordant with gear-based assessments. PLoS ONE 19: e0313170. https://doi.org/10.1371/journal.pone.0313170

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. Molecular Ecology 21(8): 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Yamanaka H, Minamoto T, Matsuura J, Sakurai S, Tsuji S, Motozawa H, Hongo M, Sogo Y, Kakimi N, Teramura I, Sugita M, Baba M, Kondo A (2017) A simple method for preserving environmental DNA in water samples at ambient temperature by addition of cationic surfactant. Limnology 18(2): 233–241. https://doi.org/10.1007/s10201-016-0508-5

Supplementary material 1

Summary of the eDNA water sampling from the Boso and Motobu Peninsulas

Authors: Shin-ichiro Oka, Tetsuya Sado, Masayuki Ushio, Masaki Miya

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.135461.suppl1

Supplementary material 2

Lists of deep-sea fish (top) and purely freshwater fish (bottom) detected from the Boso and Motobu Peninsulas

Authors: Shin-ichiro Oka, Tetsuya Sado, Masayuki Ushio, Masaki Miya

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.135461.suppl2

Supplementary material 3

List of fish species detected by the eDNA metabarcoding from 72 samples (3 sample bags × 3 sites × 4 rounds of sampling × 2 peninsulas) from the Boso and Motobu Peninsulas

Authors: Shin-ichiro Oka, Tetsuya Sado, Masayuki Ushio, Masaki Miya

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.135461.suppl3

Supplementary material 4

Summary of indicator taxa analysis results showing bias in species occurrence between the outgoing and incoming tides

Authors: Shin-ichiro Oka, Tetsuya Sado, Masayuki Ushio, Masaki Miya

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.135461.suppl4

Supplementary material 5

Venn diagrams showing the results of variation partitioning analysis at each site

Authors: Shin-ichiro Oka, Tetsuya Sado, Masayuki Ushio, Masaki Miya

Data type: tiff

Link: https://doi.org/10.3897/mbmg.8.135461.suppl5

Supplementary material 6

Species accumulation curves with 95% CI of the fish community at each site

Authors: Shin-ichiro Oka, Tetsuya Sado, Masayuki Ushio, Masaki Miya

Data type: tiff

Link: https://doi.org/10.3897/mbmg.8.135461.suppl6

Supplementary material 7

The R code used for the variation partitioning analysis

Authors: Shin-ichiro Oka, Tetsuya Sado, Masayuki Ushio, Masaki Miya

Data type: zip

Explanation note: When the compressed file is extracted, you will obtain the R code used for variation partitioning analysis and the data used in the analysis.

Link: https://doi.org/10.3897/mbmg.8.135461.suppl7